

1 THIS IS THE UNCORRECTED VERSION ACCEPTED FOR PUBLICATION IN APPETITIE. THE PAPER WAS
2 PUBLISHED IN [VOLUME 150](#), 1 JULY 2020, 104653 AND CAN BE FOUND
3 <https://doi.org/10.1016/j.appet.2020.104653>

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5 **Is breastfeeding associated with later child eating behaviours?**

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42

43 **Abstract**

44 Individual differences in children’s eating behaviours emerge early. We examined the relationship
45 between breastfeeding exposure and subsequent eating behaviours among children from the Growing
46 Up in Singapore Towards healthy Outcomes (GUSTO) cohort. Children (n = 970) were grouped according
47 to their breastfeeding exposure: high (full breastfeeding \geq 4 months with continued breastfeeding \geq 6
48 months), low (any breastfeeding < 3 months or no breastfeeding) and intermediate (between low and
49 high breastfeeding categories). Aspects of eating behaviour from ages 15 months to 6 years were
50 captured using a combination of maternal reports (Child Eating Behaviour Questionnaire; Infant Feeding
51 Questionnaire; Preschooler Feeding Questionnaire) and laboratory-based measures of meal size, oral
52 processing behaviours (e.g. average eating speed and bite size) and tendency to eat in the absence of
53 hunger. Most children had low (44%) or intermediate (44%) breastfeeding exposure; only 12% had high
54 exposure. After adjusting for confounders, multivariable linear regression analyses indicated the high
55 (but not intermediate) breastfeeding group was associated with significantly lower reported food
56 fussiness at 3 years compared to low breastfeeding group (-0.38 [-0.70, -0.06]), with similar but non-
57 significant trends observed at 6 years (-0.27 [-0.66, 0.11]). At 3 years, mothers in the high breastfeeding
58 group also reported the least difficulty in child feeding compared to low breastfeeding group (-0.22 [-
59 0.43, -0.01]). However, high breastfeeding was not associated with any other maternal-reports of child
60 feeding or eating behaviours, and no significant associations were observed between breastfeeding
61 exposure and any of the laboratory measures of eating behaviour at any of the time points. These
62 results do not strongly support the view that increased breastfeeding exposure alone has lasting and
63 consistent associations with eating behaviours in early childhood.

64

65 **Keywords:**

66 Breastfeeding; Child eating behaviours; Oral processing; Satiety responsiveness: Food fussiness

67

68 **Abbreviations:**

69 AGA, appropriate for gestational age; BF, Breastfeeding; CEBQ, Child Eating Behavioural Questionnaire;
70 EAH, Eating in the Absence of Hunger; IFQ, Infant Feeding Questionnaire; LGA, large for gestational age;
71 PFQ, Preschooler Feeding Questionnaire; SGA, small for gestational age.

72 **1. Introduction**

73 Early childhood is an important period for establishing eating behaviours that underpin good nutrition
74 and healthy growth (WHO, 2016). Behaviours such as a slower eating speed (Berkowitz et al., 2010;
75 Fogel et al., 2017b), responsivity to satiety cues (Carnell, Benson, Gibson, Mais, & Warkentin, 2017) and
76 consumption of a variety of fruits and vegetables (Boeing et al., 2012) have been associated with later
77 weight outcomes in childhood, with individual differences in these behaviours emerging early and
78 shaped in part by the feeding environment (Kral et al., 2018). As breastfeeding is the first type of feeding
79 that many infants are exposed to, it is suggested to be an early target for promoting healthier eating
80 behaviours from a young age (Taveras et al., 2004).

81

82 There are several pathways through which breastfeeding has been hypothesised to influence a child's
83 eating behaviour. First, the sensory experience of human breastmilk might shape early food
84 preferences through flavour learning (Mennella, 2014). A variety of tastes and flavour volatiles ingested
85 by the mother during pregnancy are transmitted to the foetus via the amniotic fluid, which continues
86 postpartum through breastmilk feeding to the infant (Mennella, 2014; Mennella & Trabulsi, 2012).
87 Randomised studies have reported that infants are more accepting of a carrot-flavoured food during
88 weaning if their mother consumed carrot juice whilst breastfeeding, compared to infants whose
89 mothers consumed water (Mennella & Beauchamp, 1999; Mennella, Daniels, & Reiter, 2017; Mennella,
90 Jagnow, & Beauchamp, 2001). Others have found breastfed infants are more rapid in their acceptance of
91 novel foods and flavours compared to those who are formula fed (Maier, Chabanet, Schaal, Leathwood,
92 & Issanchou, 2008), and continue to accept a wider variety of vegetables at age 15 months, 3 and 6
93 years (Maier-Nöth, Schaal, Leathwood, & Issanchou, 2016) and reported to be less fussy at age 7 years
94 (Galloway, Lee, & Birch, 2003). This suggests an important role of breastmilk feeding in the
95 development of children's early food preferences and acceptance beyond specific flavour learning
96 (Forestell & Mennella, 2007; Hausner, Nicklaus, Issanchou, Mølgaard, & Møller, 2010; Sullivan & Birch,
97 1994).

98

99 Compositional features of breastmilk have also been linked to the development of appetite control
100 mechanisms in infancy. Unlike formula milk, breastmilk changes in composition from one feed to
101 another and across the lactation period (Ballard & Morrow, 2013). Breastfed infants may therefore have
102 more opportunity to adapt nutrient intake in response to a fluctuating macronutrient content (Mitoulas
103 et al., 2007; Woolridge, Ingram, & Baum, 1990) and appetite-related hormones present in human

104 breastmilk (Ballard & Morrow, 2013; Newburg, Woo, & Morrow, 2010; Savino & Liguori, 2008).
105 Additionally, the act of breastfeeding is reported to encourage a more responsive and less controlling
106 feeding style in mothers (Blissett & Farrow, 2007; Farrow & Blissett, 2006; Taveras et al., 2004), which
107 may promote eating self-regulation through mothers being more aware of their child's satiety cues and
108 infants gaining more control over their own feeding times and the volume of milk they are exposed to.
109 In support, longer breastfeeding duration has been associated with increased parent reported satiety
110 responsivity in toddlers (Brown & Lee, 2012) and a lesser tendency to eat in the absence of hunger in
111 adolescents (Reyes et al., 2014), suggesting that breastfeeding experience might impact children's
112 appetite regulation beyond the breastfeeding period.

113
114 Despite the multiple pathways through which breastfeeding may impact children's eating behaviour,
115 consistent evidence to support an enduring relationship between breastfeeding exposure and eating
116 behaviours later in childhood is still lacking, particularly for the proposed link to eating behaviours
117 thought to represent better self-regulation of energy intake. To address this, we examined the
118 relationship between breastfeeding duration and a variety of child eating behaviours, captured as a
119 combination of maternal-reports and laboratory-based measures assessed between ages 15 months and
120 6 years in the multi-ethnic Growing Up in Singapore Towards healthy Outcomes (GUSTO) study. Faster
121 eating and the tendency to snack in the absence of hunger are two behaviours that have been
122 consistently associated with increased energy intake in a laboratory meal setting in the GUSTO cohort
123 (Fogel et al., 2017b; Fogel, McCrickerd, et al., 2018). Faster eating was also associated with higher BMI
124 and adiposity at 4.5 years (Fogel et al., 2017b) and mothers' reports of lower satiety responsivity at 6
125 years (Fogel, Fries, et al., 2018). If breastfeeding experience supports the development of eating
126 behaviours in this cohort, we expect that children with greater exposure to breastfeeding might be less
127 fussy in their eating styles, but also exhibit eating behaviours that support better energy intake
128 regulation, such as higher satiety responsivity, slower eating, smaller meal size and less eating in the
129 absence of hunger. Evaluating the link between breastfeeding and a variety of eating behaviours within
130 the same cohort should help clarify whether public health advice to increase breastfeeding exposure can
131 optimise the development of eating behaviours in early childhood.

132

133 **2. Methods**

134 ***2.1 Study design and population***

135 In 2009-2010, women in their first trimester of pregnancy were recruited from the maternity units of
136 two major Singaporean public hospitals, KK Women's and Children's Hospital and the National
137 University Hospital. Women were eligible to join the "Growing Up in Singapore Toward healthy
138 Outcomes" (GUSTO) study if they were of Chinese, Malay or Indian ethnicity with homogenous parental
139 ethnic background, between 18-46 years old, not diagnosed with type I diabetes mellitus and not on any
140 psychotropic medications or undergoing chemotherapy. Details of the cohort (n = 1247) have been
141 published previously (Soh et al., 2014). Written informed consent was obtained from all participants,
142 and the study (NCT01174875; <https://clinicaltrials.gov/>) was granted ethical approval by both the
143 National Healthcare Group Domain Specific Review Board and SingHealth Centralized Institutional
144 Review Board.

145

146 A flow chart of participants included in the current study is presented in Figure 1. Only singleton
147 children born at term (≥ 37 weeks gestation) with information on breastmilk feeding exposure were
148 considered as eligible participants for this study (n = 970). Children were included based on the
149 availability of eating behaviour data from ages 15 months to 6 years. Restricted visit time availability
150 meant that only a subset participated in the eating behaviour tasks conducted at 4.5 and 6 years (see
151 Fogel, McCrickerd, et al., 2018). The final sample sizes considered for data analyses across the various
152 tasks are shown in Figure 1.

153

154 **2.2 Participant characteristics**

155 Participant baseline characteristics, including educational attainment, maternal age, ethnicity and family
156 history of atopy, were collected during the first trimester of pregnancy through interviewer-
157 administered questionnaires. Maternal pre-pregnancy BMI (kg/m^2) was derived from maternal height
158 measured at 26-28 weeks gestation (using SECA 213 Stadiometer) and pre-pregnancy weight reported
159 by the women at 11-14 weeks gestation. Information on child sex, parity and birth weight was extracted
160 from medical records. Infants were classified into birth-weight-for-gestational-age percentiles according
161 to the method described by Mikolajczyk et al. (Mikolajczyk et al., 2011), and infants who were $<10^{\text{th}}$
162 percentile were considered small-for-gestational-age (SGA), $10\text{-}90^{\text{th}}$ percentile were appropriate-for-
163 gestational-age (AGA), and $>90^{\text{th}}$ percentile were large-for-gestational-age (LGA). Child's weight and
164 length/height were measured at 12 months and 4, 5 and 6 years using a SECA 334 (12 months) or SECA
165 803 Weighing Scale (4-6 years), and a SECA 210 Mobile Measuring Mat (12 months) or SECA stadiometer

166 213 (4-6 years). Child BMI z-scores were derived using an R macro provided by the World Health
167 Organization (WHO, 2006).

168

169 **2.3 Breastfeeding exposure**

170 Using interviewer-administered questionnaires, breastfeeding practices, including whether the child was
171 still breastfed and to what extent, were captured as 'exclusive', 'predominant', 'partial' breastfeeding or
172 'formula feeding', based on WHO breastfeeding definitions (WHO, 2008; WHO/UNICEF, 1993). The
173 questionnaire was conducted with each mother at every postnatal visit starting from week 3, then at 3-
174 monthly intervals from month 3 to month 18 (when mothers were asked to record responses for every
175 month within the 3-month interval between visits), and then yearly intervals from year 2 to year 4.

176

177 Full breastfeeding was initially defined as exclusive or predominant breastfeeding at each time point
178 (Pang et al., 2016), and this was used to categorise children as having experienced 'low', 'intermediate'
179 or 'high' breastfeeding exposure according to previously defined criteria (Cai et al., 2015). High-
180 breastfeeding infants were those fully breastfed for 4 months who continued to breastfeed at least
181 partially until at least 6 months. Intermediate-breastfeeding infants were breastfed at least partially
182 beyond 3 months but did not meet the criteria for high breastfeeding. Low-breastfeeding infants were
183 weaned from breastmilk or exclusively formula-fed before the age of 3 months. Based on these
184 definitions, breastfeeding exposure includes infants who were both fed at the breast and through
185 expressed breastmilk.

186

187 **2.4 Outcome measures: Children's eating behaviours**

188 2.4.1 Maternal-reports of feeding practices and child eating behaviours at 15 months, 3 and 6 years

189 Three established parent-report questionnaires assessing aspects of maternal feeding beliefs and
190 practices and child eating behaviours were administered at different ages: the Infant Feeding
191 Questionnaire (IFQ; Baughcum et al., 2001), the Preschooler Feeding Questionnaire (PFQ; Baughcum et
192 al., 2001), and the Children's Eating Behaviour Questionnaire (CEBQ; Wardle, Guthrie, Sanderson, &
193 Rapoport, 2001). All questionnaires were completed by the mothers. To focus the study outcomes, only
194 subscales that were measured at multiple time points and specifically related to aspects of children's
195 food fussiness, acceptance of new foods, satiety responsivity and tendency towards overeating were
196 included.

197

198 The IFQ was completed when the children were 15 months old. The IFQ has seven subscales used to
199 assess a mother's feeding beliefs and practices. Previous validation of the IFQ in the GUSTO cohort has
200 shown a moderately good fit with the original 7-subscale structure hence the original classification of
201 the items in the IFQ was mostly adopted (Quah et al., 2016). Two subscales (*Awareness of infant's*
202 *hunger and satiety cues* and *Concern about infant overeating or becoming overweight*) were analysed.
203 The scores for each subscale, captured as the averages of their item scores, were used as outcome
204 variables.

205
206 The PFQ was completed when the children were 3 years old. Of the eight PFQ subscales, two (*Difficulty*
207 *in child feeding* and *Concern about child overeating or being overweight*) were analysed. Subscale
208 scores, averaged from item scores contributing to the subscale, were used as outcome variables in the
209 analyses.

210
211 Mothers also completed the CEBQ when the children were 3 and 6 years old. The CEBQ captures eight
212 parent-reported appetitive traits, which can be broadly categorized as either food-approach and food-
213 avoidance behaviours (Wardle et al., 2001) and has been validated in the GUSTO cohort (Quah et al.,
214 2017; Quah et al., 2019); of the revised subscales suitable for this cohort, *Satiety responsiveness* and
215 *Food fussiness* were included in the current analyses using z-scores generated by Quah et al. as outcome
216 variables.

217

218 2.4.2 Observed eating behaviours at ages 4.5 and 6 years

219 In addition to maternal reports, several observed eating behaviours were assessed during *ad libitum*
220 lunches served at both 4.5 and 6 years of age. The procedures for these meal observations have been
221 published in detail elsewhere (Fogel et al., 2017a; Fogel, McCrickerd, et al., 2018). The children were
222 fasted for a minimum of three hours before each lunch session, which took place in the same test room
223 at both time points and contained child-appropriate furniture and utensils. The sessions were recorded
224 with three high-resolution CCTV cameras positioned to capture the child's behaviour from multiple
225 angles. The mother was present in the room during the 4.5-year visit and was requested to interact with
226 her child as normal but not to interfere with the child's food choices or intake. Mothers were not
227 present in the lunch room at the 6-year time point.

228

229 2.4.2.1 *Ad libitum* Energy intake

230 During the lunch session, children were served an *ad libitum* meal consisting of foods and drinks
231 selected to be familiar and relatively well-liked, based on responses to a Food Frequency Questionnaire
232 (FFQ) collected at an earlier time point. At 4.5 years, children were served a buffet-style lunch consisting
233 of 9 foods and 3 drinks: white bread (Gardenia; 2.63 kcal/g; 6 slices), pancakes (Aunty Jemima; 3 kcal/g;
234 70 g), Honey Stars cereal (Nestle; 3.8 kcal/g; 80 g), chocolate cake (Sara Lee; 4.3 kcal/g; 80 g), cheese
235 (Cowhead; 2.95 kcal/g; 66 g), chicken nuggets (CP; 2.29 kcal/g; 216 g), chicken cocktail sausage
236 (Fairprice; 2.95 kcal/g; 192 g), apple slices (0.44 kcal/g, 204 g), canned corn (Hosen; 0.81 kcal/g; 160 g),
237 apple juice (Marigold; 0.5 kcal/ml; 6 boxes), full cream milk (Marigold; 0.65 kcal/ml; 6 boxes) and water.
238 At 6 years, children were presented with a self-served meal consisting of vegetarian fried rice (800 g;
239 1.86 kcal/g) and water.

240
241 At both time points, children were instructed to serve themselves from the portions provided and eat as
242 much as they liked for their lunch. Additional portions of all the foods were provided by the research
243 staff if any were completely consumed. Children consumed their lunch within 30 minutes and rated
244 their hunger/fullness before and after lunch using a similar five-point picture rating scale ranging from
245 'Hungry' to 'Very full' at 4.5 years and 'Very hungry' to 'Very full' at 6 years. The foods, including any
246 leftovers, were weighed before and after consumption using a balance accurate to 0.1 g and this was
247 used to estimate energy consumed during lunch (Fogel, McCrickerd, et al., 2018).

248 249 *2.4.2.2 Eating rate and oral processing behaviours*

250 Children's eating rate and oral processing behaviours during lunch were determined by coding the CCTV
251 videos using behavioural annotation software ELAN (version 4.9.1, Max Planck Institute for
252 Psycholinguistics, The Netherlands), in combination with an established coding scheme (Fogel et al.,
253 2017a). The frequency of each bite, chew and swallow taken throughout the meal was recorded and
254 used to determine the total time (in sec) spent with food in the mouth (total oral exposure time) and
255 without (inter-bite interval). This was then combined with the weight of the foods consumed during the
256 meal to derive the average eating rate (g/min), bite size (g/bite), chews per gram and total meal
257 duration (min) for each of the meals. The video-coding was performed by a single trained coder. A
258 random 10% of the videos within each time point were validated by a second trained coder and showed
259 excellent inter-rater reliability (intra-class correlation coefficients were 0.954 and 0.995 at 4.5 and 6
260 years, respectively). Among all children who participated in the lunch session at year 6, we excluded

261 from the analyses children who had insufficient oral processing data due to very short mealtimes (n =
262 43).

263

264 *2.4.2.3 Eating in the absence of hunger (EAH)*

265 Children completed an EAH task at both 4.5 and 6 years, following a 'free access' protocol adapted from
266 Fisher & Birch (2002), approximately 10 minutes after lunch was cleared. Children were provided with
267 colouring paper and crayons and encouraged to play by the researcher. After 5 minutes, the researcher
268 placed two sweet (M&M: 18 units, 4.83 kcal/g; Hello Panda: 10 units, 5.43 kcal/g) and two savoury
269 (Rollercoster: 10 units, 5.55 kcal/g; Want Want; 2 units 4.83 kcal/g) snacks separately in small bowls on
270 the table within the child's reach, and informed the child that the researcher would be leaving the room
271 to prepare the next activity and that he or she could have the snacks if they wished. The researcher
272 returned after 5 minutes and collected the bowls before taking the child to the next task. The snacks
273 were weighed before and after to estimate energy intake.

274

275 Among the children who completed the EAH tasks, those who consumed < 50g of food at lunch and
276 indicated that they were still hungry afterwards were excluded from statistical analyses. This approach is
277 in line with previous works (Fisher & Birch, 2002; Fogel, Fries, et al., 2018), as the absence of hunger
278 could not be assumed for these children (n = 82 at 4.5 years and n = 108 at 6 years).

279

280 **2.5 Statistical analyses**

281 Statistical analyses for this study was prespecified to examine the association of breastfeeding exposure
282 (low, intermediate or high breastfeeding) with children's eating behaviours from maternal-report
283 questionnaires, and with energy intake, eating rate and the oral processing behaviours derived from the
284 lunch tasks using multivariable linear regression. As some children did not eat in the absence of hunger
285 during the EAH task, children were categorized as 'No' vs 'Yes' for EAH; logistic regression was used to
286 examine the relationship between breastfeeding and EAH. For all adjusted models, we selected
287 confounders based on previous publications (Brown & Lee, 2012; de Barse et al., 2017), and included
288 maternal age (years), ethnicity (Chinese, Malay, Indian), maternal education (secondary and below,
289 technical school or tertiary), maternal pre-pregnancy BMI (kg/m²), child sex and birth weight category
290 (small for gestational age (SGA), appropriate for gestational age (AGA), large for gestational age (LGA)).
291 For the EAH tasks, models were also adjusted for energy intake (kcal) during the lunch task that
292 preceded it.

293

294 Several sensitivity analyses were performed, some of which are provided as supplemental material.
295 First, we explored the effect of additional adjustment for parity (primiparous, multiparous) and family
296 history of allergy (yes, no) for outcomes related to food fussiness (de Barse et al., 2017), and results are
297 presented in Supplementary Table 2. Second, as the error residuals of the observed eating behaviour
298 variables at 4.5 and 6 years were not normally distributed, we log-transformed these outcome variables;
299 similar results were obtained (results not shown). Third, we examined the reproducibility of results
300 among children who had a complete set of eating behaviour outcomes across all time points, *i.e.* from
301 15 months to 6 years of age ($n = 109$; see Supplementary Table 3). All patterns of data were the same.
302 Finally, we examined whether ethnicity or child sex modified the association of breastfeeding and eating
303 behaviours by including interaction terms in the regression analyses; there appears to be no significant
304 modification effect with all p -interaction ≥ 0.08 (see Supplementary Table 5). Adjustment for multiple
305 comparisons was not performed as it increases the type II error for the associations that are not null
306 (Rothman, 1990). Participants (0-8.7%) with missing covariates were excluded from the statistical
307 analyses. All statistical analyses were performed using IBM SPSS (Version 25).

308

309 **3. Results**

310 ***3.1 Study and participant characteristics***

311 Participant characteristics (overall and by breastfeeding exposure group) are summarized in Table 1. The
312 majority of study children had either low or intermediate exposure to breastfeeding during infancy
313 (44.3% and 43.5%, respectively); only 12.2% had high breastfeeding exposure. Mothers who were
314 younger, of Malay ethnicity, with lower educational attainment or with higher pre-pregnancy BMI were
315 more likely to be in the low breastfeeding group. Child BMI Z-scores from 1 to 6 years were similar
316 across the different breastfeeding exposure groups.

317

318 As differing numbers of participants were included in the analyses for various eating behaviour tasks and
319 questionnaires, we examined participant characteristics for each subset (Supplementary Table 1). We
320 found no significant differences among participants who were included and excluded for the year 4.5
321 lunch task analyses, or among those with or without a complete set of outcome data. However, mothers
322 who were not included in the month 15 IFQ analyses were significantly younger and of Malay ethnicity,
323 with a lower proportion of Chinese ethnicity. For year 3 CEBQ analyses, a larger proportion of Chinese
324 and a lower proportion of Malay mothers were excluded from the analyses compared to those included.

325 Similarly, more Chinese and less Malay mothers, and those with lower BMI were excluded from the year
326 6 CEBQ analyses. For year 6 lunch tasks, younger and a larger proportion of Indian mothers were not
327 included in the analyses.

328

329 ***3.2 Maternal-reported child feeding and eating behaviours at 15 months and 3 and 6 years***

330 The unadjusted mean (\pm SD) and adjusted mean differences in maternal reports of child feeding and
331 eating behaviours across the breastfeeding exposure groups are shown in Table 2 (additional regression
332 details are shown in Supplementary Table 4). Compared to mothers in the low breastfeeding group,
333 those in the intermediate breastfeeding group reported greater awareness of their infant's hunger and
334 satiety cues at 15 months and were least concerned with their child overeating/being overweight at 3
335 years old. However, the same trends were not seen in the higher breastfeeding group, nor were there
336 any differences in reported concern of infant overeating/overweight between breastfeeding exposure
337 groups at the earlier time point. At 3 years, mothers in the high breastfeeding group reported less
338 difficulty in child feeding than those in the low breastfeeding group. This association became statistically
339 non-significant with further adjustment for parity and family history of allergy, although its direction was
340 similar (see Supplementary Table 2).

341

342 We found no significant associations between breastfeeding exposure and the CEBQ measure of satiety
343 responsiveness at 3 or 6 years (Table 2). However, children in the high breastfeeding group had
344 significantly lower levels of reported food fussiness at 3 years than those in the low breastfeeding group;
345 this trend was weaker and no longer significant at age 6 years (see Table 2). Similar differences in
346 reported food fussiness seen at both 3 and 6 years remained after further adjustment for parity and
347 family history of allergy in the additional sensitivity analysis but were no longer statistically significant
348 (Supplementary Table 2).

349

350 ***3.3 Observed eating behaviours***

351 The unadjusted mean (\pm SD) and adjusted mean differences in observed laboratory-based eating
352 behaviours are also presented in Table 2. Across children exposed to different levels of breastfeeding,
353 we found no significant differences in oral processing behaviours, energy consumed during lunch or
354 tendency to EAH at ages 4.5 or 6 years.

355

356 **4. Discussion**

357 Early childhood is an important period for establishing children’s eating behaviours. Breastfeeding has
358 been hypothesized to support the development of food preferences and appetite control mechanisms
359 during this period through several pathways. Our data from the GUSTO cohort show only a few
360 associations between breastfeeding exposure during infancy and a variety of maternal-reports of child
361 feeding and laboratory-based measures of eating behaviours captured during the preschool years. These
362 findings thus do not strongly support the view that increased breastfeeding exposure alone is likely to
363 confer a substantial advantage to children’s health through lasting and consistent associations with
364 eating behaviours in childhood.

365
366 Aspects of children’s eating behaviours were captured using a combination of maternal reports and
367 objective measures of laboratory-based food intake. Across these measures, maternal reports of food
368 fussiness (CEBQ) and difficulty in child feeding (PFQ) were the only variables to show consistent links
369 with prior breastfeeding exposure. The highest exposure to breastfeeding was significantly associated
370 with less food fussiness when the children were 3 years old, with similar but non-significant differences
371 observed when the children were 6 years old. This is consistent with previous studies linking longer
372 breastfeeding duration with less food fussiness at age 7 (Galloway et al., 2003) and increased
373 acceptance of a variety of foods during the preschool years (Forestell & Mennella, 2007; Hausner,
374 Nicklaus, Issanchou, Molgaard, & Moller, 2010; Maier et al., 2008). However, relationships were
375 weakened after age 3 years in the current cohort and further attenuated after accounting for parity,
376 suggesting that the positive effect of increased breastfeeding exposure on ease of feeding and food
377 fussiness may be overridden over time within the family environment. For the majority of children, a
378 developmentally appropriate phase of fussy or picky eating tends to peak around 3 years (Cardona Cano
379 et al., 2015), and it is possible that any influence of prior breastfeeding exposure may also be strongest
380 within this timeframe.

381
382 Despite some consistency in the trend for maternal reports of food fussiness, there were no other
383 consistent links between increasing breastfeeding exposure and maternal reports of awareness of
384 satiety cues, concerns about overeating or later satiety responsiveness. The few other studies assessing the
385 link between breastfeeding exposure and parent reports of eating behaviours also lack in consistency.
386 For example, Brown and Lee (2012) reported better CEBQ-reported satiety responsiveness among 18-24
387 month-old children who were breastfed for longer. By contrast, Rogers and Blissett (2017) reported that
388 longer breastfeeding duration was not significantly associated with CEBQ-reported satiety

389 responsiveness or food fussiness at 12 months. Several factors could explain these mixed findings. First,
390 studies have differed in their definition of breastfeeding exposure; some examined breastfeeding
391 duration and exclusivity separately in relation to eating behaviours (e.g. Brown & Lee, 2012), while
392 others examined breastfeeding exclusivity for different duration (e.g. Rogers & Blissett, 2017) and we
393 integrated both the duration and exclusivity of breastfeeding when assessing associations with child
394 eating behaviours. Finally, this research spans a variety of ages, from as young as age 12 months (e.g.
395 Rogers & Blissett, 2017) to 6 years in our study. The interpretation of a child's behaviour captured in
396 parent-report measures such as the CEBQ may be more stable in older children (Quah et al., 2019).
397 When children are younger, such as 12 and 15 months, these measures may be more indicative of
398 maternal behaviours and feeding practices than the child's eating behaviours *per se*.

399
400 Our direct observations of children's eating behaviours at 4.5 and 6 years arguably provide more
401 objective measures than those captured in maternal-report questionnaires. Faster eating speed, energy
402 intake at a meal and the tendency to eat in the absence of hunger have been identified as behavioural
403 markers of children's eating self-regulation (Kral et al., 2018) and satiety responsivity (Carnell & Wardle,
404 2007). In the GUSTO cohort, faster eating speed (characterized by larger bite sizes and less chewing) has
405 been shown to predict increased energy intake at a meal, child adiposity and weaker satiety
406 responsiveness as reported by the mother (Fogel, Fries, et al., 2018; Fogel et al., 2017a). Similarly, higher
407 levels of EAH at 4.5 years predicted increased EAH, larger portion selection and increased energy intake
408 at 6 years in the same cohort of children (Fogel, McCrickerd, et al., 2018). However, we found no
409 significant association of breastfeeding exposure with eating behaviours at age 4.5 and 6 years, nor a
410 consistent trend with child BMI from age 4 to 6 years (Table 1). By contrast, the one previous study
411 linking breastfeeding exposure to later observations of self-regulatory eating behaviours reported that
412 shorter breastfeeding duration was associated with an increased likelihood of reporting hunger after a
413 meal and EAH by 16-17 year old Chilean adolescents (Reyes et al., 2014). Methodological differences,
414 such as the ages at which eating behaviours were measured, a limited overlap in the specific eating
415 behaviours captured and socio-cultural variations between Asian young children and Chilean
416 adolescents, may contribute to these different findings. Taken together, these data highlight that more
417 longitudinal evidence is required to better understand and characterise the impact, if any, of
418 breastfeeding exposure on later child eating behaviour.

419

420 Strengths of our study include its longitudinal nature, with various aspects of children’s eating
421 behaviours captured over time, particularly those related to energy intake control. Statistical models
422 were also adequately adjusted for, with information collected on relevant confounders. However, there
423 are some limitations to the current study that may restrict the conclusions drawn. Firstly, not all
424 mothers and children were included in all the measures at every time point. Where possible, sensitivity
425 analyses were conducted to corroborate the study findings and account for the variable participant
426 numbers. Another important consideration is that our measure of breastfeeding exposure included
427 infants who were fed expressed breastmilk from a bottle, and therefore we cannot discern the effects of
428 the different modes of breastmilk feeding (i.e. infants fed at the breast vs those fed breastmilk by the
429 bottle) on subsequent eating behaviours. It is possible that the self-regulatory characteristics of eating
430 behaviour, such as satiety responsivity and eating speed, may relate less to the duration of breastmilk
431 exposure and more to the mode of breastmilk feeding, as suggested by previous studies (Disantis,
432 Collins, Fisher, & Davey, 2011; Li, Fein, & Grummer-Strawn, 2010; Ventura & Hernandez, 2019). Small
433 sample sizes in the GUSTO cohort meant that the mode of breastmilk feeding on children’s ability to
434 self-regulate food intake could not be concurrently explored in the current dataset, however this will be
435 considered independently in future work. More research is required to understand the interplay
436 between breastfeeding duration and the mode of breastmilk feeding, and their links to subsequent
437 parental feeding practices and child eating behaviours.

438

439 In conclusion, our results suggest that greater exposure to breastfeeding during infancy is not strongly
440 associated with eating behaviours linked to energy intake regulation in childhood, but showed some
441 small associations with increased maternal reports of food fussiness in the preschool years. Despite its
442 other documented health benefits, greater breastfeeding exposure seems unlikely to have a major
443 impact on later eating behaviours in childhood.

444

445 **Funding source**

446 This research was supported by the Singapore National Research Foundation under its Translational and
447 Clinical Research (TCR) Flagship Programme and administered by the Singapore Ministry of Health’s
448 National Medical Research Council (NMRC), Singapore - NMRC/TCR/004-NUS/2008; NMRC/TCR/012-
449 NUHS/2014. KMG is supported by the UK Medical Research Council (MC_UU_12011/4), the National
450 Institute for Health Research (as an NIHR Senior Investigator (NF-SI-0515-10042) and through the NIHR
451 Southampton Biomedical Research Centre) and the European Union’s Erasmus+ Capacity-Building ENeA

452 SEA Project and Seventh Framework Programme (FP7/2007-2013), projects EarlyNutrition and ODIN
453 under grant agreement numbers 289346 and 613977. Additional funding was provided by the Singapore
454 Institute for Clinical Sciences, Agency for Science Technology and Research (A*STAR), and Abbott
455 Nutrition Research and Development Asia-Pacific Center.

456

457 **Conflict of Interest Disclosures**

458 KMG, LPS, CGF and Y-SC have received reimbursement for speaking at conferences sponsored by
459 companies selling nutritional products. KMG, S-YC and Y-SC are part of an academic consortium that has
460 received research funding from Abbott Nutrition, Nestec and Danone. CGF currently serves on the
461 scientific advisory council for Kerry Taste and Nutrition. The other authors have no financial or personal
462 conflict of interest to declare.

463

464 **Acknowledgments**

465 We wish to thank participants of the GUSTO study, all staff involved in the collection and management
466 of data, and the GUSTO study group. The GUSTO study group includes Allan Sheppard, Amutha
467 Chinnadurai, Anne Eng Neo Goh, Anne Rifkin-Graboi, Anqi Qiu, Arijit Biswas, Bee Wah Lee, Birit F.P.
468 Broekman, Boon Long Quah, Borys Shuter, Chai Kiat Chng, Cheryl Ngo, Choon Looi Bong, Christiani
469 Jeyakumar Henry, Claudia Chi, Cornelia Yin Ing Chee, Yam Thiam Daniel Goh, Doris Fok, E Shyong Tai,
470 Elaine Tham, Elaine Quah Phaik Ling, Evelyn Chung Ning Law, Evelyn Xiu Ling Loo, Fabian Yap, Falk
471 Mueller-Riemenschneider, George Seow Heong Yeo, Helen Chen, Heng Hao Tan, Hugo P S van Bever,
472 Iliana Magiati, Inez Bik Yun Wong, Ivy Yee-Man Lau, Izzuddin Bin Mohd Aris, Jeevesh Kapur, Jenny L.
473 Richmond, Jerry Kok Yen Chan, Joanna D. Holbrook, Joanne Yoong, Joao N. Ferreira., Jonathan Tze Liang
474 Choo, Jonathan Y. Bernard, Joshua J. Gooley, Keith M. Godfrey, Kenneth Kwek, Kok Hian Tan,
475 Krishnamoorthy Niduvaje, Kuan Jin Lee, Leher Singh, Lieng Hsi Ling, Lin Lin Su, Ling-Wei Chen, Lourdes
476 Mary Daniel, Lynette P Shek, Marielle V. Fortier, Mark Hanson, Mary Foong-Fong Chong, Mary Rauff,
477 Mei Chien Chua, Melvin Khee-Shing Leow, Michael Meaney, Mya Thway Tint, Neerja Karnani, Ngee Lek,
478 Oon Hoe Teoh, P. C. Wong, Paulin Tay Straughan, Peter D. Gluckman, Pratibha Agarwal, Queenie Ling
479 Jun Li, Rob M. van Dam, Salome A. Rebello, Seang-Mei Saw, See Ling Loy, S. Sendhil Velan, Seng Bin Ang,
480 Shang Chee Chong, Sharon Ng, Shiao-Yng Chan, Shirong Cai, Shu-E Soh, Sok Bee Lim, Stella Tsotsi, Chin-
481 Ying Stephen Hsu, Sue Anne Toh, Swee Chye Quek, Victor Samuel Rajadurai, Walter Stunkel, Wayne

482 Cutfield, Wee Meng Han, Wei Wei Pang, Yap-Seng Chong, Yin Bun Cheung, Yiong Huak Chan and Yung
483 Seng Lee.

484

485 **Author contributions**

486 Y-SC, KMG, FY, KHT, S-YC, LPS, MJM, JGE designed and led the GUSTO study; MSK, Y-SC, WWP
487 conceptualized and designed the current study; DF, WWP, MCC, SBL, AF, KM, IMA, WLY conducted
488 research; WWP, AF, KM, IMA, WLY, PLQ provided essential data; WWP performed statistical analysis;
489 WWP, KM wrote the first draft of manuscript; MSK, S-YC, KMG, AF, IMA, WLY, PLQ, MEW, JGE, CGF,
490 MCFF critically reviewed the manuscript. All authors read and approved the final manuscript; WWP and
491 KM had primary responsibilities for final content.

492

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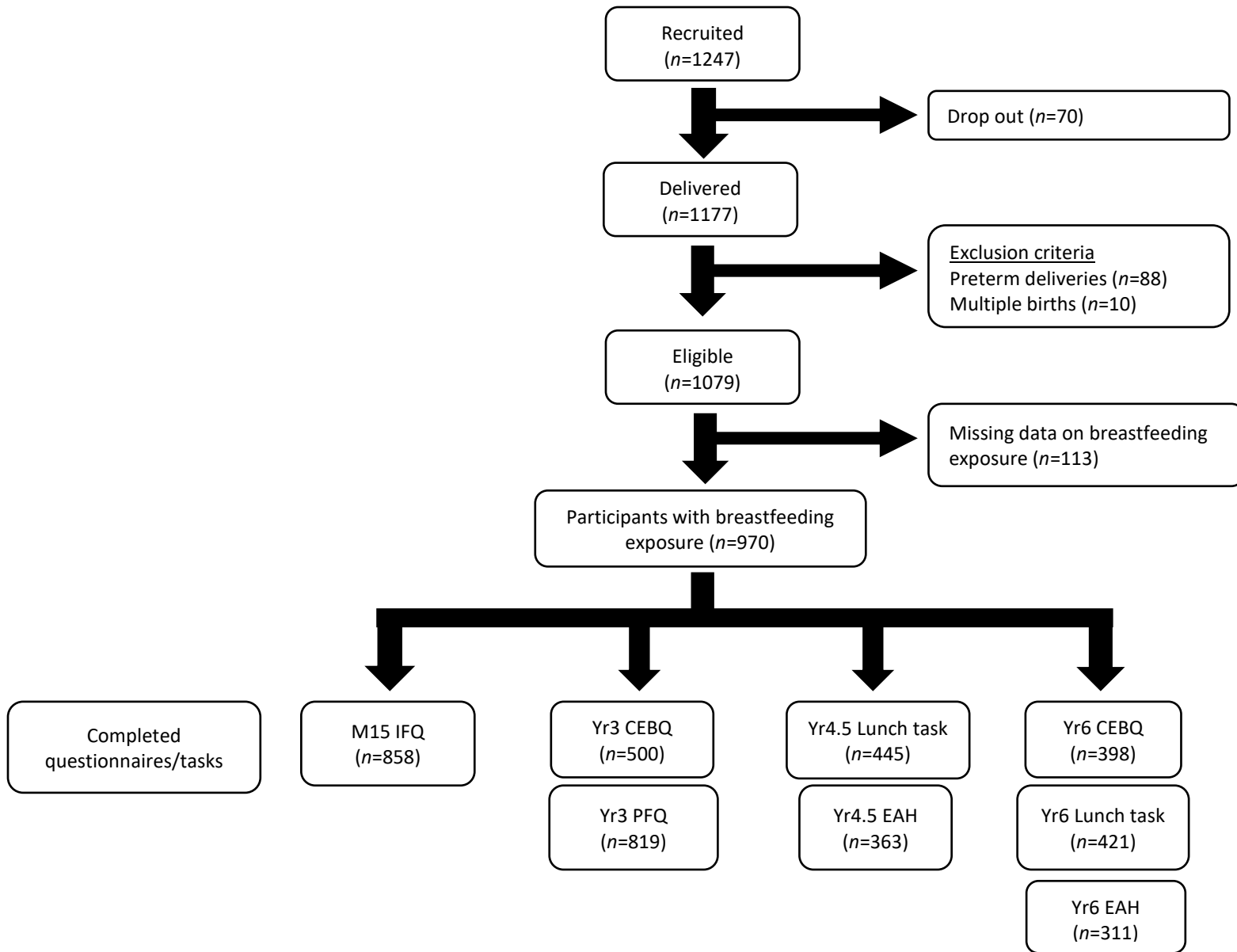


Figure 1. Flowchart of participants included in the current analyses.

Table 1. Mother-child characteristics among participants with data on breastfeeding (BF) exposure.

Participant characteristics	ALL participants (n=970)	Participants with data on breastfeeding exposure (n=970)		
		BF low (n=430, 44.3%)	BF intermediate (n=422, 43.5%)	BF high (n=118, 12.2%)
Maternal age (yr)^a	30.8 ± 5.1	29.6 ± 5.5	31.8 ± 4.7	31.3 ± 4.3
Ethnicity^c				
Chinese	558 (57.5)	206 (47.9)	262 (62.1)	90 (76.3)
Malay	239 (24.6)	146 (34.0)	85 (20.1)	8 (6.8)
Indian	172 (17.7)	77 (17.9)	75 (17.8)	20 (16.9)
Maternal education^c				
Secondary and below	285 (29.4)	192 (44.7)	76 (18.0)	17 (14.4)
Technical school	330 (34.0)	171 (39.8)	138 (32.7)	21 (17.8)
Tertiary	344 (35.5)	62 (14.4)	205 (48.6)	77 (65.3)
Maternal pre-pregnancy BMI (kg/m²)^{a,c}	22.7 ± 4.4	23.4 ± 5.1	22.3 ± 3.8	21.7 ± 3.1
Child sex				
Female	465 (47.9)	210 (48.8)	197 (46.7)	58 (49.2)
Parity				
Primiparous	441 (45.5)	197 (45.8)	195 (46.2)	49 (41.5)
Family history of allergy^c				
Yes	343 (49.9)	155 (50.0)	137 (47.2)	51 (58.6)
Child birth weight category^c				
SGA	122 (12.6)	64 (14.9)	46 (10.9)	12 (10.2)
AGA	683 (70.4)	293 (68.1)	301 (71.3)	89 (75.4)
LGA	158 (16.3)	68 (15.8)	73 (17.3)	17 (14.4)
Child BMI Z-scores at 12 months^{b,c}	-0.17 (-0.82, 0.51)	-0.07 (-0.83, 0.65)	-0.24 (-0.79, 0.39)	-0.19 (-0.92, 0.62)
Child BMI Z-scores at 4 years^{b,c}	-0.02 (-0.61, 0.70)	0.08 (-0.58, 0.89)	-0.10 (-0.64, 0.55)	0.00 (-0.64, 0.68)
Child BMI Z-scores at 5 years^{b,c}	-0.09 (-0.78, 0.67)	0.04 (-0.78, 0.87)	-0.20 (-0.81, 0.53)	-0.21 (-0.74, 0.51)
Child BMI Z-scores at 6 years^{b,c}	-0.18 (-0.91, 0.63)	-0.11 (-0.95, 0.85)	-0.25 (-0.91, 0.49)	-0.06 (-0.81, 0.61)
Duration of any breastfeeding (months)^{b,c}	3.4 (0.8, 9.2)	0.7 (0.3, 1.1)	6.8 (4.0, 11.9)	14.4 (11.8, 21.0)
Duration of full breastfeeding (months)^{b,c}	0.5 (0.5, 0.5)	0.5 (0.5, 0.5)	0.5 (0.5, 0.5)	6.0 (5.0, 6.0)

Values presented are number of participants (percentage) unless otherwise indicated.

^aValues are mean ± SD

^bValues are median (IQR)

^cNumber of participants with missing data: Ethnicity (n=1); Maternal education (n=11); Maternal pre-pregnancy BMI (n=84); Family history of allergy (n=283); Child birthweight category (n=7); Child BMI Z-scores -12M (n=90); Child BMI Z-scores- Yr4 (n=191); Child BMI Z-scores- Yr5 (n=184); Child BMI Z-scores- Yr6 (n=218); Duration of any breastfeeding (n=34); Duration of full breastfeeding (n=48).

Abbreviations: BF, breastfeeding; SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age.

Table 2. Association of breastfeeding (BF) exposure group with maternal-reports of child feeding and observational measures of eating behaviour from age 15 months to 6 years.

	N	Mean ± SD			Adjusted mean differences (95% CI) ^c		
		BF Low	BF Intermediate	BF High	BF Low	BF Intermediate	BF High
Maternal-report measures:							
Month 15							
Aware of infant's hunger & satiety cues (IFQ) ^e	858	3.3 ± 0.7	3.4 ± 0.6	3.3 ± 0.6	REF	0.1 (0.0, 0.2) ^d	0.0 (-0.2, 0.2)
Concern about infant overeating/overweight (IFQ) ^e	838	1.0 ± 0.8	0.8 ± 0.7	0.9 ± 0.8	REF	-0.1 (-0.2, 0.0)	0.0 (-0.2, 0.2)
Year 3							
Difficulty in child feeding (PFQ) ^e	819	1.6 ± 0.9	1.7 ± 0.9	1.5 ± 0.7	REF	0.1 (-0.1, 0.2)	-0.2 (-0.4, 0.0) ^d
Concern about child overeating/overweight (PFQ) ^e	813	0.9 ± 0.8	0.7 ± 0.6	0.7 ± 0.6	REF	-0.2 (-0.3, -0.1) ^d	-0.1 (-0.3, 0.1)
Satiety responsiveness (CEBQ, z-scores)	500	-0.09 ± 1.03	-0.03 ± 0.93	0.41 ± 1.03	REF	-0.03 (-0.24, 0.17)	0.24 (-0.07, 0.55)
Food fussiness (CEBQ, z-scores)	500	-0.06 ± 0.98	0.08 ± 1.02	-0.24 ± 1.04	REF	0.04 (-0.17, 0.25)	-0.38 (-0.70, -0.06) ^d
Year 6							
Satiety responsiveness (CEBQ, z-scores)	398	-0.05 ± 1.02	0.06 ± 0.98	-0.17 ± 0.85	REF	0.08 (-0.16, 0.33)	-0.11 (-0.51, 0.28)
Food fussiness (CEBQ, z-scores)	398	-0.05 ± 1.10	0.04 ± 0.99	-0.29 ± 0.73	REF	0.08 (-0.16, 0.32)	-0.27 (-0.66, 0.11)
Laboratory-based measures:							
Year 4.5							
Total lunch consumed (kcal)	445	275.8 ± 161.8	306.7 ± 152.5	315.2 ± 150.7	REF	16.6 (-18.4, 51.6)	11.8 (-39.1, 62.8)
Bite size (g/bite)	352	2.0 ± 1.3	1.9 ± 1.1	1.9 ± 1.3	REF	-0.1 (-0.4, 0.2)	-0.1 (-0.5, 0.3)
Chews per gram	352	11.0 ± 7.3	9.8 ± 5.5	10.4 ± 8.0	REF	-1.1 (-2.9, 0.6)	-0.6 (-3.2, 2.0)
Eating rate (g/min)	352	6.9 ± 3.4	6.9 ± 3.0	7.1 ± 3.5	REF	-0.2 (-1.0, 0.6)	0.3 (-0.9, 1.5)
Total meal duration (min)	352	20.9 ± 6.4	19.7 ± 6.7	20.9 ± 6.7	REF	-1.5 (-3.1, 0.1)	-0.8 (-3.3, 1.6)
EAH (yes/no intake) ^{a,b}	363	REF	1.23 (0.76, 1.97)	2.31 (1.07, 4.96) ^d	REF	1.03 (0.59, 1.79)	1.46 (0.62, 3.45)
Year 6							
Total lunch consumed (kcal)	421	196.2 ± 194.5	180.2 ± 120.3	212.6 ± 125.9	REF	-10.1 (-46.4, 26.1)	-3.2 (-58.5, 52.1)
Bite size (g/bite)	421	3.9 ± 3.3	3.7 ± 2.2	3.6 ± 1.2	REF	-0.1 (-0.6, 0.4)	-0.3 (-1.1, 0.4)
Chews per gram	421	5.2 ± 3.2	5.3 ± 3.0	4.3 ± 1.8	REF	0.3 (-0.4, 1.0)	-0.5 (-1.5, 0.5)
Eating rate (g/min)	421	13.4 ± 12.3	12.5 ± 7.7	13.8 ± 5.2	REF	0.2 (-1.7, 2.1)	1.4 (-1.4, 4.3)
Total meal duration (min)	421	10.8 ± 5.8	10.7 ± 6.2	10.7 ± 6.1	REF	-0.1 (-1.5, 1.3)	-1.4 (-3.5, 0.8)
EAH (yes/no intake) ^{a,b}	311	REF	1.27 (0.65, 2.51)	1.71 (0.55, 5.35)	REF	1.17 (0.51, 2.66)	1.70 (0.43, 6.78)

^aResults are presented as odds ratio (95% CI).

^bModels were additionally adjusted for total lunch consumed (kcal).

^cModels adjusted for maternal age (years), ethnicity (Chinese, Malay, Indian), maternal education (secondary and below, technical school, tertiary), maternal pre-pregnancy BMI, child sex, child birthweight category (SGA, AGA, LGA).

^dValues are $P < 0.05$ compared to the reference group.

^eMean subscale scores ranged from 0 to 4.

Abbreviations: EAH, eating in the absence of hunger.